BACTERICIDAL EFFECT OF ULTRAVIOLET RADIATION

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The effects of ultraviolet radiation on microorganisms have been studied frequently. The lethal action of sunlight on certain bacteria was demonstrated by Downes and Blunt as early as 1877. Roux (1887) showed that spores as well as bacteria are destroyed by these radiations and in 1903 Barnard and Morgan reported that the bactericidal action of radiant energy is limited to wavelengths shorter than 3000Å.

The susceptibility of microorganisms to ultraviolet radiation under varying conditions has also received considerable attention. Wells and Wells (1936), Whiser (1940), and Koller (1939) claim that air-borne bacteria are ten times as resistant to radiation at high relative humidity as when floating in air at low humidity. Koller's results indicate that floating bacteria are less resistant to ultraviolet than similar bacteria on the agar surface of a Petri plate and Wells (1940) states that air-borne bacteria are about twenty times less resistant than those floating in a liquid suspension. Rentschler and Nagy (1940) report the same sensitivity for air borne and for identical bacteria on the surface of agar and no effect due to humidity. The Bunsen-Roscoe reciprocity law for the bactericidal action of ultraviolet has been tested by Coblentz and Fulton (1924), by Gates (1929) and by Koller (1939) with varying results. Conflicting theories have been advanced to explain the nature of the bactericidal action of ultraviolet radiation.

In recent years a rapid and simple method for measuring ultraviolet radiation has been made available by one of us (Rent-

schler, 1930). This has stimulated a search for a better understanding of the action of radiation on bacteria and other microorganisms.

The object of this paper is to present the results obtained from an extensive investigation of the bactericidal action of ultraviolet radiation. All tests were made quantitative, in that the amount of effective radiation applied to the bacteria was measured for every single exposure. These experiments have an important bearing on the theories advanced for the action of radiation on microorganisms and the results are of definite value in the practical use of ultraviolet radiation as a bactericidal and fungicidal agent.

ULTRAVIOLET METER

The simple integrating ultraviolet meter used in these experiments was first described in 1930 (Rentschler, 1930). For measuring the bactericidal radiation, a photocell with tantalum (Rentschler, Henry and Smith, 1932) as the active material was used; this element responds only to radiations shorter than 3000 Å (that is to bactericidal ultraviolet).

To measure the integrated amount of radiation, a battery, B, charges a condenser, C, through the photocell, P, at a rate depending upon the effective intensity of the radiation falling on P, and the response of the cell (fig. 1). A special glow tube, G, having a thorium cathode b and two anodes a and d provides a simple means for discharging the condenser, C, and automatically recording the number of times it is charged during an exposure. When the condenser, C, is charged to the breakdown potential between a and b, a glow from a to b discharges it, thereby starting the main current from d to b. (The spacing between d and b is such that the full battery voltage is not sufficient to ignite the discharge from d to b by itself.) The large condenser, K, charged through a high resistance r, thus discharges through a relay, R, thereby operating a recorder or a counter. (The resistance r is sufficiently high so that the battery, B, does not maintain the discharge from d to b after the condenser, K, is discharged.)

The number of discharges for a given exposure represents in

arbitrary units, (counter clicks), the effective photoelectric current through the cell, P, and, therefore, the integrated effective radiation to which the cell was exposed; the rate of discharging represents the average intensity of the radiation. In the work here described a mechanical counter was used for recording the discharges. A less automatic method has recently been suggested by Taylor (1939) to take the place of the special glow tube and the counter.

In measuring the small photocurrents produced by low intensity radiation, the leakage over the glass surface or across the condenser in humid weather causes serious errors. This leakage was eliminated by mounting the cell elements in one and the con-

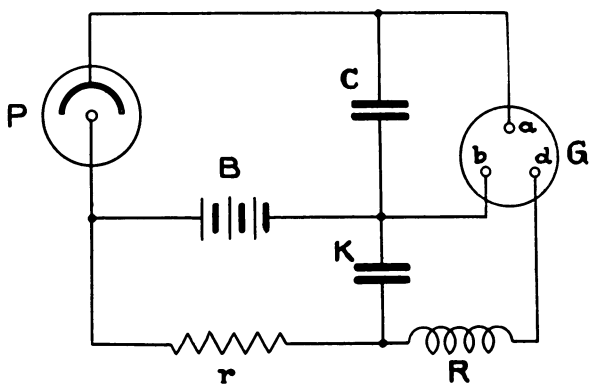


FIG. 1. ULTRAVIOLET METER CIRCUIT

denser and glow tube in the other of a two compartment bulb as shown in figure 2. A cell so constructed is reliable for charging periods of thirty minutes or more, thus enabling one to measure very low intensities with accuracy. Both compartments are exhausted to eliminate the possibility of the formation of a conducting film. Such cells are constant over years of operation and are easily constructed.

The cell with the condenser and glow tube was calibrated so that each discharge or click of the meter expressed the bactericidal effectiveness of the radiation in terms of microwatt seconds per sq. cm. of 2537 Å radiation from mercury. For this calibration

the photocell was placed at a fixed distance in front of a spirally-coiled low pressure quartz mercury discharge lamp with a quartz water cell between the lamp and the photocell. The time required for one click was determined. The intensity of the radiation in microwatts per sq. cm. was measured by a thermopile, first through a quartz and then through a barium-flint glass

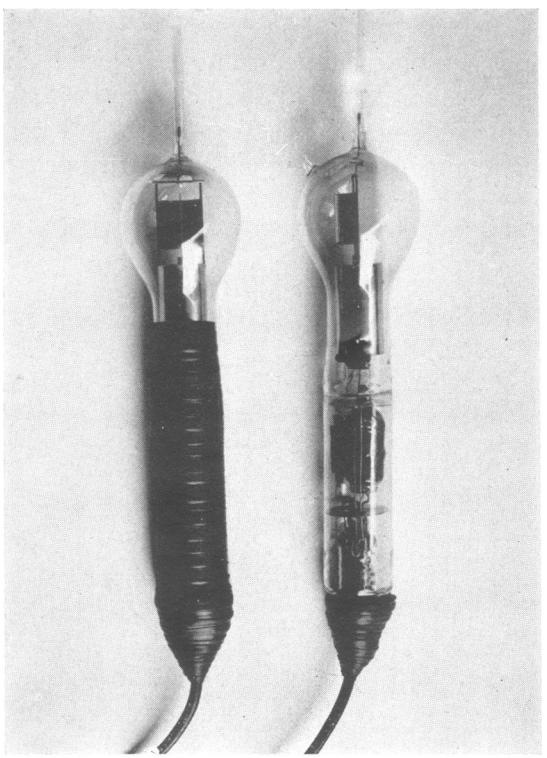


FIG. 2. PHOTOCELL UNIT

filter. The difference between the two values, corrected for reflections from the filters, determines the radiation intensity below 3000 Å and since about 98 per cent of this is concentrated in the 2537 Å line it may be considered as radiation of this wavelength. The thermopile was calibrated with an incandescent lamp standardized by the National Bureau of Standards. The value of the "click" for the meter used in the tests reported in this paper was

thus determined to be 220 microwatt seconds per sq. cm. of radiation at 2537 Å wavelength. The radiation measurements from any light source are simpler to determine in terms of clicks than in microwatt seconds per sq. cm. For this reason all measurements in this paper are recorded in clicks.

SEEDING PETRI PLATES WITH BACTERIA

The problem of determining the bactericidal action of ultraviolet radiation was greatly simplified by the use of a unique method for seeding bacteria on the surface of agar in Petri plates. As an illustration twenty ml. of a 1:200 dilution of a 24-hour broth culture of *Escherichia coli* was atomized into a closed box five feet on a side by means of an ordinary paint spray gun at about 50 pounds pressure from a nitrogen tank. The spray was allowed to settle for two minutes to separate out the larger floating drops

TABLE 1

Number of colonies of E. coli on Petri plates simultaneously seeded by the spray method

1280	1260	1278	1275	
1293	1290	1268	1272	
1268	1285	1284	1250	Avg. 1275

and to permit the turbulence of the air inside to subside. A dozen or more open sterile agar Petri plates on a tray were then placed on the floor of the box. About 1200 bacteria settled on the surface of the agar in each Petri plate in the next four minutes. A group of plates thus simultaneously seeded and incubated develop the same under of colonies within $\pm$ 3 per cent of the average. The uniformity of the colony count of such a test seeding is shown in table 1.

This method of seeding is safe with non-pathogenic bacteria and was found most helpful in establishing the factors controlling the effects of ultraviolet radiation on bacteria, yeasts and molds.

DETERMINATION OF THE BACTERICIDAL ACTION OF ULTRAVIOLET RADIATION

The tantalum photocell and the seeded Petri plate to be irradiated were placed side by side at a distance of about eighteen

TABLE 2

Per cent of E. coli killed by ultraviolet radiation expressed in meter clicks

TEST NUMBER	AGE CULTURE	CULTURE	SPRAY	SETTLING TIME	SEEDING TIME	COLONIES ON CONTROL	2	4	7	10	13	17	20	24	28	32
	hours	*cc.*	*cc.*	*min.*	*min.*											
1	21	.0025	20	1	4	570	—	31	45	66	79	90	94	98.6	99.2	100.
2	21					566	5	27	54	67	86	90	96.7	99.	99.4	99.9
3	24					640	9	39	54	71	84	—	95.6	98.4	99.8	99.8
4	23					700	23	43	63	81	88	—	95.	98.4	99.3	99.7
5	27					668	25	35	59	72	83	92.6	95.7	98.	99.6	100.
6	24					640	36	45	62	66	88	94.	96.7	99.1	99.5	99.7
7	24					543	30	50	52	—	77	91	91	97.5	98.5	99.2
8	24					530	19	59	65	79	—	93.	96	97.	99.7	99.8
9	22					165	15	32	44	66	79	83	85			
10	23					1125	18	—	55	68	75	85	91.3			
11	22		80	1	6	322	38	60	78	76	87	91	91.9			
12	23					375	29	50	64	76	83	96	92.			
13	22					653	20	46	69	79	87	93	96.			
14	24					350	39	59	77	85	85	—	96			
15	24					655	29	45	77	82	90	95	98			
16	25	.005	160	3	10	730	31	39	73	75	85	89	93	97	97.3	98.3
17	25					460	30	51	70	86	93	94	95	98.6	99.1	99.5
18	23					343	27	—	71	79	87	94	95			
							29									
							29									
							26									
19	23					288	20	63	70	75	83	97	96			
									63							
									74							
20	23					510	21	41	61	79	82	91	96			
								41								
								43								
								49								
21	22					755	8	24	53	70	78	90	94			
							11									
							10									
							16									

22	22					220	26	52	65	80	87	95	98			
									64							
									67							
									60							
23	22					712	14	36	60	68	80	92	97			
								39								
								28								
								37								
24	23					317	16	45								
							21	41								
							24	45								
							22	46								
25	23					785	15	40								
							21	44								
							19	41								
							16	38								
							17	37								
26	5.5					118	26	58	75	86	95	97	99			
27	6.5					108	24	66	81	89	93	94	97			
28	7					110	28	40	70	77	79	96	94			
29	7.5					103	31	50	74	87	90	91	94			
30	8					120	23	58	73	85	93	93	99			
31	3	.1	20	2	4	1130	38	59	77	88	93	97	99.8	99.4	99.8	99.9
32	2					952	50	62	86	95	96	98.5	98.8	100.	99.9	99.9
33	4					922	35	65	89	91	97	98.5	99.8	99.8	100.	100.
34*	24	.5	20	4	1	2989	58	60	70	70	75	84	91	97	99.	99.6
35*	24					1780	25	36	57	67	63	75	88	97	98	—
36†	24	.005	20	2	4	1117	32	49	60	72	84	94	98	98.5	99.8	99.9
37‡	20	.005	160	3	10	309	28	64	80	87	90	93	94			
38‡	17					181	37	64	74	88	94	93.4	93	96	97	98.5
39§	24	.0025	20	2	4	970	2	5	22	24	42	53	55	66	78	81

* Tests #34 and #35 were seeded at 98–100 per cent relative humidity.
† Test #36—Culture was ground with sand for 25 minutes before spraying.
‡ Tests #37 and #38—Plates were incubated at 37°C. for 2 hours after seeding and before exposing.
§ Test #39—Plates incubated at 37°C. for 5.5 hours after seeding and before exposing.

TABLE 3

Per cent of various organisms killed by ultraviolet radiation expressed in meter clicks

TEST NUMBER	AGE CULTURE	CULTURE	SPRAY	SETTLING TIME	SEEDING TIME	COLONIES ON CONTROL	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	40	45	50	55	60	65	70
						Pseudomonas fluorescens																											
	hours	*cc.*	*cc.*	*min.*	*min.*																										
40	24	.0025	20	2	4	355	8	27	62	80	90			95		96		98		99.4		100									
41						355	23	27	57	79	89			94		97		98		98.6		100									
42						1200	4	22	53	70	83			91		93		95		98		98.7									
43						2290	8	14	41	61	69			79		87		92		96		98									
						Proteus vulgaris																											
44	24	.0025	20	2	3	308	19	50	76	88	95.5			97.8		97.5		98.7		99.3		99.7									
45						270	40	55	83	94	95			98.5		99		99.2		99.6		99.6									
46						1877	30	49	66	78	83			90		93		95		97		98.3									
47						1740	16	63	63	77	85			91		94		96		97		98.8									
						Phytomonas tumefaciens																											
48	24	.0025	20	2	2	640			35		56			78		86		92		96			97	98		99.3	99.9				
49						1077			34		64			80		92		94		98.5			99.5	99.7		99.9	99.9				
50						597			25		54			72		88		94		97			98.8	99.6		99.9	99.9				
51						1180			25		61			70		85		90		95			98.5	99.		99.8	99.9				
52						670			23		57			76		93		96		96			99.6	99.2		99.9	100				
53						757			26		65			80		92		94		98			99.2	99.7		99.8	100				
54	24	.1	20	2	4	2520			29		57			80		81		90		96			97.6	98.5		99.7	99.				
						Bacillus fusiformis																											
55	24	.1	20	2	5	647	13	14	56	76				92		96		97		99.2			99.9	100							
56						532	36	46	77	86				93		97		98		99.4			99.6	100							

Serratia marcescens

57	24	.0025	20	2	4	2350	19	65	77	90	95	97	98.5	99.4	99.9	99.9						
58						767	15	25	83	93	97	98.5	99.2	99.5	100.	100.						
59						878	43	77	91	97	98	99.3	99.9	99.9	99.9	99.9						
60						2130	19	63	88	93	98	99.1	99.5	99.9	100	100						

Bacillus subtilis

61	24	1	20	2	5	230		5	53		37	66		71	71		76	75	82	85		
62	24	2	20	2	5	369		25	21		30	53		68	64		76	81	85	88		
63	24	.5	20	2	4	895		15	38		66	80		86	94		96	98	98	99		
64	24	.1	20	4	3	110		26	45		59	66		87	93		99	98	100	100		

Streptococcus lactis

65	24	.005	20	1	2	497		15	11		13	42		66	89		97	99				
66						528		2	9		22	55		92	92		99	99.9				
67						924		15	21		41	71		89	98		99	100				

Neisseria catarrhalis

68	24	.0025	20	2	4	815		10	33		68	84		94	97			98.5		99.5	100	
69						794		27	75		86	94		97	98			99.4		100	99.9	
70						1035		39	78		91	96		98	97			99.3		99.9	100	

Staphylococcus albus

71	24	.0025	20	2	3	692	6	15	40	70	85	96		99.3		99.9						
72						728	18	19	53	67	88	97		99.4		100						

Micrococcus piltonensis

73	24	.0025	20	2	4	270		13	32		36	56.8		63	68			81		95	99.3	
74						301		20	65		—	44		56	67			85		94	99.6	
75						765		—	16		30	44		59	72			95		98	—	

Micrococcus spheroides

76	24	.1	20	2	4	637		—	17		14	28		26	32			50		—	94.5	97
77						780		29	35		17	30		44	56			65		81		
78						848		11	6		25	38		47	51			70			96	99.9

inches from an ultraviolet lamp. Unless otherwise specified, the ultraviolet radiation was produced by a discharge through a mixture of inert gases with mercury vapor at low pressure in a special high-ultraviolet-transmitting glass tube.[1] In each test two plates were used as controls and the remaining exposed individually to different amounts of radiation measured by the meter and expressed in clicks. All the plates of a test were incubated for 48 hours and the colonies on the controls and exposed plates counted. From the number of colonies on the various plates the percentage of the bacteria killed by the different amounts of radiation was calculated. Similar measurements were also made for various yeasts and molds. These will be published elsewhere. The results of some tests on various bacteria under different conditions are shown in tables 2, 3 and 4.

In different tests the variation in the lethal action on *E. coli* by the same amount of radiation is wider than can be attributed to non-uniformity of seeding (see table 1). This variation is most marked for low exposures such as 2, 4, and 7 clicks. That the variation, likewise, cannot be attributed to errors in exposure can be seen from Tests 18 to 25. In any one of these tests the percentage of bacteria killed on a number of simultaneously seeded plates and exposed to the same amount of radiation is, within experimental error, the same.

It was anticipated that an appreciable number of the colonies may originate from droplets each containing several bacteria, thus causing a difference in the lethal action. Tests 1 to 25 with various dilutions of the spray-mixture do not bear out this assumption.

It was suspected that the age of the culture had an influence on the resistivity of the bacteria to ultraviolet radiation. This was tested by spraying and exposing very young cultures of *E. coli* (Tests 26–33). Also, Petri plates seeded with 24-hour cultures were incubated for 2 hours before exposing to the ultraviolet (Tests 37 and 38). The individual bacteria in a culture are not all at the same stage of their life cycle (by life cycle of a

[1] The lamp used was a Westinghouse "Sterilamp."

TABLE 4

Per cent of various organisms killed by ultraviolet expressed in terms of meter clicks

TEST NUMBER	AGE CULTURE	CULTURE	SPRAY	SETTLING TIME	SEEDING TIME	COLONIES ON CONTROL	5	10	20	30	40	50	60	70	80	90	100	110	120
							Sarcina lutea												
	hours	*cc.*	*cc.*	*min.*	*min.*														
79	24	.05	20	3	10	243				11	10	27	46	75	87		97		98.7
80						300				2.6	1.3	35	44	74	77		90		99.3
81						294					42	49	64	65	88		93		95
82	24	.1	25	3	10	1400				9.1	18	26	36	68	74		92		96
83*	24					1304			12		27	43	60	62	78		96		98
84†	24					1782			18		39	68	79	76	94		98		99
85‡	24	.1	25	3	4	972		16		23	35	41	57	69	85	87	95	98	
86§	25	.1	25	2	4	1382		15		39	47	64	82	88	96	99	99.1	100	
87¶	24	.1	20	2	4	2067	13	26		48	63	84	91	98	96	99.6	99.4		
							B. subtilis **(spores)**												
88	1 wk.	5	20	2	5	2570		16	26	31	48	62	69		93				
89	1 wk.		20	2	5	540		12	33	60	80	87	95	96	95	94			
90	1 wk.		20	2	5	967		21	41	77	74	92	95	97	99	99.6	99.8		
							Spirillum rubrum												
91		1 loop	20	1	4	392			25	63	97.8	99.4							
92	24 hrs.	.1	20	1	4	88	20	88	97.8	98.8	100								
93	24 hrs.	.1	20	1	4	88	27	78	99	100	100								

* Test #83 is the same as #82 except that the culture was shaken 30 min. before seeding.

† Test #84 is the same as #82 except culture was placed on a diaphragm vibrating 60 cycles for one-half hour to break up packets.

‡ Test #85 same as #84 except vibrated for 70 min.

§ Test #86: The culture was ground 4 minutes with sand and then sprayed.

¶ Test #87 same as #86 excepting ground for 70 minutes.

bacterium is meant the period from the instant it is formed by subdivision to the time it itself divides). The age of the culture may markedly influence the relative distribution of the number of bacteria in the various stages of the life cycle. The wide variation in the apparent sensitivity of the different cultures follows at once if it is assumed that the same individual bacterium has a markedly different resistance to ultraviolet radiation in successive phases of its life cycle. This theory is further verified from the study of the Bunsen-Roscoe reciprocity law for the bactericidal action of ultraviolet radiation for low intensities as shown later in this paper. It will also be shown that in addition to the variation due to the different stages of the life cycle, individual bacteria from the same cultures may vary in resistivity to radiation. Thus, in the same culture of *E. coli* at the most resistant stage of the life cycle, the lethal radiation for the individual bacteria may differ appreciably.

A further complication arises when applying radiation to such bacteria as streptococci, staphylococci and sarcinae where the colonies originate from clusters. Some of the individuals in the clump may shield others from the radiation. Every one of the bacteria in a cluster must be destroyed to prevent the formation of a colony. Clump formation was simulated by incubating *E. coli* seeded on a plate for about 5 hours at 37°C. The apparent lethal action (as estimated by colonies formed) is definitely less for such partially incubated plates (see Test 39). This explains some of the results obtained with bacteria in Tests 40 to 93.

EFFECT OF TEMPERATURE UPON THE BACTERICIDAL ACTION OF ULTRAVIOLET RADIATION

Twelve Petri plates were simultaneously seeded with *E. coli* in the usual manner. Four were placed in an incubator at 37°C. for 15 minutes and then exposed while in the incubator to two units (clicks) of ultraviolet radiation. Four plates were placed in a refrigerator at 5°C. for 15 minutes and exposed at 5°C. to 2 units (clicks). The remaining four plates were used as controls. All twelve plates were then incubated for 48 hours and the percentage killed in the two cases determined. The test was repeated

with *E. coli* for 4, for 7 and for 10 units (clicks) of radiation. The results given in table 5 are representative of a number of similar tests showing that the temperature of the bacteria at the time of irradiation does not materially affect resistivity to ultraviolet and that the variations in the different tests in tables 2, 3 and 4 cannot be attributed to the temperature of the organisms at the time of exposure.

TABLE 5

Effect of temperature on the resistivity of E. coli to ultraviolet radiation

EXPOSURE IN METER COUNTS	PERCENTAGE KILLED FOR	
	Plates at 37°C.	Plates at 5°C.
clicks		
2	20.8	22.4
4	40.0	28
7	51.3	59.5
10	82.9	80.6

TANTALUM PHOTOCELL FOR MEASURING THE BACTERICIDAL RADIATION FROM DIFFERENT ULTRAVIOLET SOURCES

In all the tests previously described, the same ultraviolet source was used. For such tests any simple sensitive integrating meter, which responds only to the bactericidal ultraviolet is generally satisfactory. There are, however, cases where it is impossible to maintain exactly the same spectral distribution from the radiation source. Gates (1929) showed that the lethal action of ultraviolet increases from 3000 Å to a maximum at approximately 2660 Å, then decreases to a minimum at 2375 Å, and again increases for still shorter wave length radiations. The photoelectric response of tantalum begins at 3000 Å and increases for decreasing wavelengths. A tantalum photocell in the proper ultraviolet-transmitting glass has a peak response very close to that for maximum bactericidal radiation. The ultraviolet response of such a cell is sufficiently similar to the bactericidal action so that it may be used to measure the effective bactericidal radiation from most practical ultraviolet sources. To check the bacteri-

cidal response of the tantalum photocell for radiations from different ultraviolet sources a number of Petri plates were seeded with *E. coli* in the usual manner. Some of these plates were exposed to a definite amount of radiation from one ultraviolet lamp as measured with the cell and some to the same amount of radiation from other light sources and other plates were used as controls. All the plates were incubated. The percentage killed by the same measured radiation from the various light sources is shown in table 6.

Within experimental error the percentage killed by the same amount of radiation from the different lamps as measured by the tantalum cell was the same. Thus, for all practical purposes, such

TABLE 6

Response of the tantalum photocell to bactericidal radiation from various sources

ULTRAVIOLET SOURCE	PERCENTAGE BACTERIA KILLED FOR	
	4 clicks	7 clicks
Low pressure mercury discharge in ultraviolet transmitting glass	35	70.1
Low pressure mercury discharge in quartz	36	60.5
Open arc between U carbons	46.6	71.5
A.C. Hanovia quartz mercury arc	36	59.9

a tantalum photocell was found satisfactory in evaluating the bactericidal radiation from any of the commonly used ultraviolet lamps.

RECIPROCITY LAW

A test of the Bunsen-Roscoe reciprocity law is of utmost importance for a theoretical consideration of the bactericidal action of ultraviolet radiation. With the help of the tantalum photocell it was possible to test the law over a range of intensities heretofore impossible. A $2\frac{1}{2}$ microfarad high voltage condenser, K, was charged with a small 15000 volt transformer, T, through a kenotron rectifier, R (fig. 3). A special lamp, L, was connected across the condenser, K, through a spark gap, G. When the necessary voltage was attained to break down the gap resistance, the con-

denser discharged through the lamp, L, producing an intense radiation lasting only a few microseconds.

The discharge lamp, L, with cold electrodes about 4″ apart, in high ultraviolet-transmitting glass containing Krypton at about 13 mm. pressure together with a few drops of mercury, was used in most of these tests. This combination produces appreciable bactericidal ultraviolet radiation either from the condenser discharge or when excited by a transformer. Discharges through other gases either alone or mixed with mercury were also used but these are generally less powerful or less convenient to use.

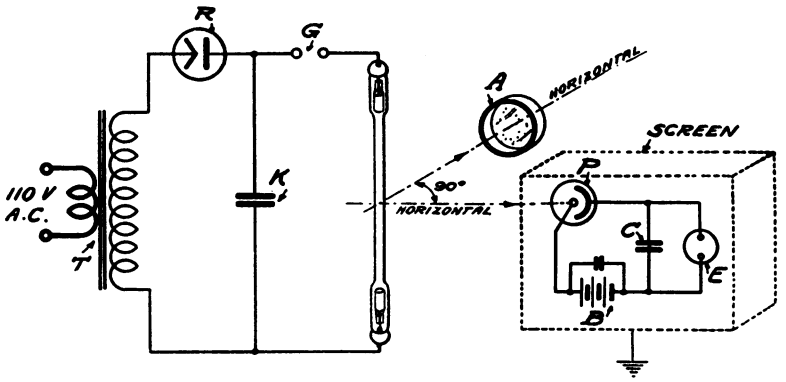


FIG. 3. CIRCUIT FOR PRODUCING AND MEASURING HIGH INTENSITY SHORT DURATION BACTERICIDAL RADIATION

A Petri-plateholder, A, was fixed about 15 inches from the lamp, L. The tantalum photocell, P, a battery, B, a suitable condenser, C, and an electrometer, E, in a grounded screen cage were connected as shown in diagram (fig. 3). The cell, P, the lamp, L, and the holder, A, remained in the same fixed positions throughout the tests. The spark gap, G, was adjusted so that the ultraviolet from a single flash discharge of the condenser, K, through the lamp, L, killed a definite percentage of *E. coli* seeded on the Petri plate placed in the holder, A. The capacity, C, was adjusted so that the photocurrent through P caused by the ultraviolet from the flash charged the condenser, C, to such a potential that the electrometer deflection was for the sake of accuracy nearly full scale. To test the reciprocity law twelve Petri plates

were simultaneously seeded with *E. coli* in the usual manner. Four plates were each exposed to a single flash through the lamp, each flash producing the same electrometer deflection. Four plates were then exposed to the ultraviolet from the same lamp excited by a low current transformer until the same electrometer deflection was obtained; these latter exposures required several minutes each. The four remaining plates were used as controls. All plates were incubated and the percentage killed determined with the results shown in table 7. These results show that the reciprocity law holds over a range of intensities covering exposures from a few microseconds to several minutes.

TABLE 7

Per cent colonies of E. coli killed by equal amounts of radiation from the flash discharge and from the transformer discharge

FLASH DISCHARGE	TRANSFORMER DISCHARGE
26.4	22
35.6	32.4
49.7	45.8
61.	63.3
79.	71.4

To test the reciprocity law further for longer exposure times ,a number of wooden holders were made with holes just large enough to insert a Petri plate. The fronts of these holders were covered with an ultraviolet-transmitting cellophane and the lower half was covered also with a sheet of metal foil. A strip of moistened blotting paper extended into the holder into the space behind the foil. The open seeded plate was inserted into the holder facing the cellophane and set in a vertical position with the bottom of the blotting paper dipping into a dish of water. In this way the plate could be exposed for a long period of time without dehydration of the agar or contamination by air suspended organisms. A photograph of the holder is shown in figure 4.

Plates from the same seeding were set up at distances of one, two, three to fifteen or more feet from a "Sterilamp" with less than one inch of the lamp exposed, so that the radiation may be

considered as coming from a point source. The photocell of the meter was placed at a fixed distance from the lamp. The upper half of the plate at one foot was exposed to a certain number of meter discharges or clicks. The plate at two feet was exposed to exactly four times this number as recorded by the meter, etc., so that the upper halves of all the plates were exposed to the same total amount of radiation but for progressively longer times. Any fluctuation in the output of the lamp due to temperature, voltage or other changes was thus corrected for by the meter.

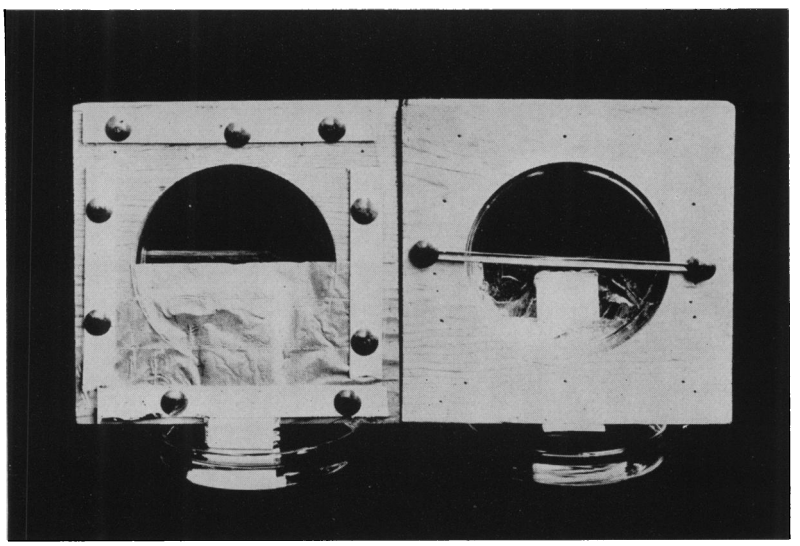


FIG. 4. PETRI PLATE HOLDER USED FOR LONG TIME EXPOSURES TO LOW INTENSITY ULTRAVIOLET

The result of exposing plates with *E. coli* in a room at 20°C. at the different distances is shown in figure 5.

The greater lethal action for the long time exposures is to be expected on the theory that the bacteria are more sensitive to radiation at certain stages of their life cycle. To test this theory further the experiment was repeated in a constant temperature room at 90°F. or 32⅔°C. At this higher temperature the life cycle of *E. coli* is shorter and the increased killing appears earlier as is shown in figure 6.

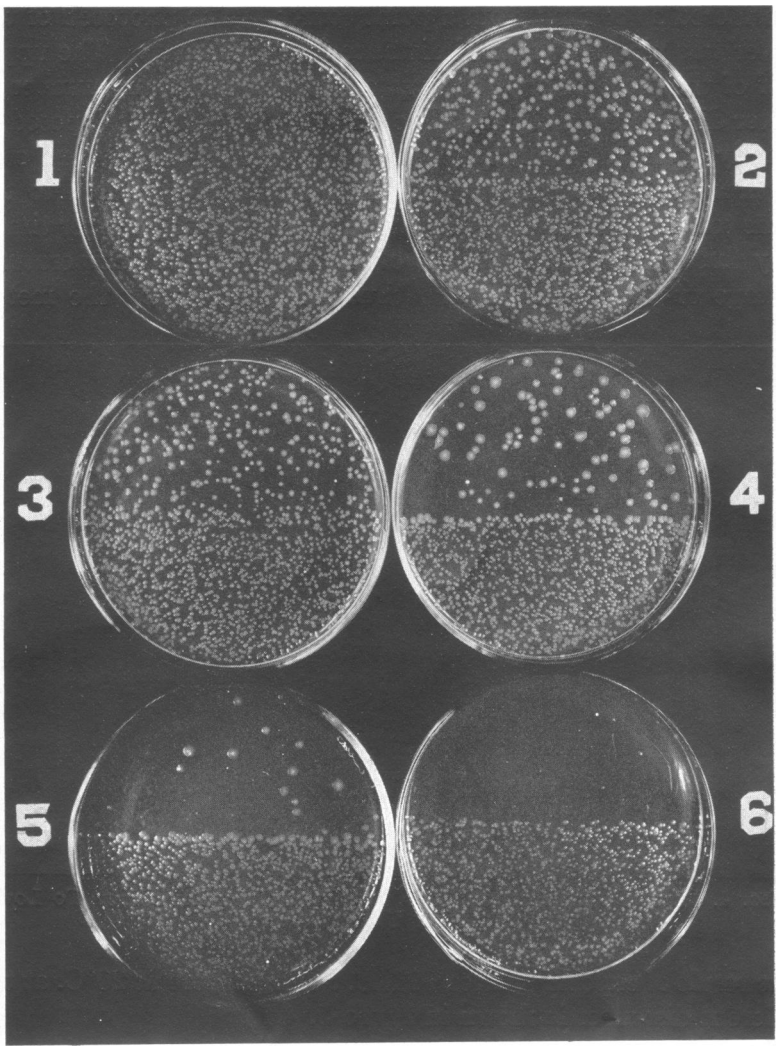


FIG. 5. E. COLI EXPOSED TO AN EQUIVALENT OF 6 CLICKS AT 20°C. AS FOLLOWS: #1, control; 2, exposed 10 minutes; 3, exposed 4 hours 17 minutes; 4, exposed 14 hours 15 minutes; 5, exposed 33 hours 15 minutes; 6, exposed 38 hours 20 minutes.

The relation between the percentage killed and the exposure times at the two temperatures for the tests of figures 5 and 6 is shown by the curves of figure 7.

The percentage colonies killed again decreases after a certain

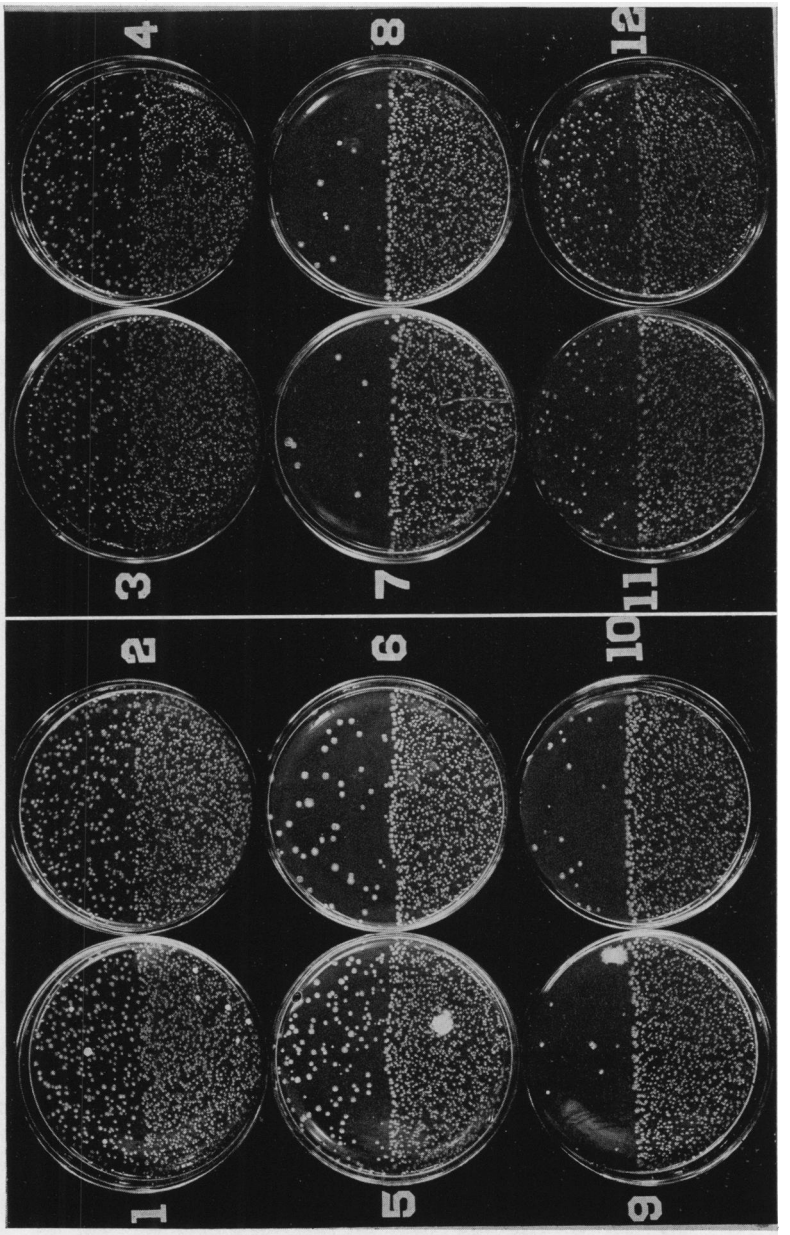


FIG. 6. E. COLI EXPOSED TO AN EQUIVALENT OF 6 CLICKS AT 32½°C. AS FOLLOWS:

#1, exposed 10 minutes; 2, exposed 40 minutes; 3, exposed 1 hour 33 minutes; 4, exposed 2 hours 43 minutes; 5, exposed 4 hours 18 minutes; 6, exposed 6 hours 20 minutes; 7, exposed 8 hours 23 minutes; 8, exposed 10 hours 58 minutes; 9, exposed 13 hours 53 minutes; 10, exposed 17 hours 8 minutes; 11, exposed 20 hours 53 minutes; 12, exposed 25 hours 23 minutes.

minimum intensity is reached for each temperature. The rate of division of the more resistant organisms under this condition is more rapid than the destruction by the radiation; this results in an apparent decrease in percentage colonies destroyed by the radiation.

As further evidence of the difference in the sensitivity of the organisms at various stages of the life cycle the following test was made. Twelve plates were seeded with *E. coli* in the usual manner. Some of the plates were incubated for various lengths of time at 37°C. before exposure to ultraviolet. The results are

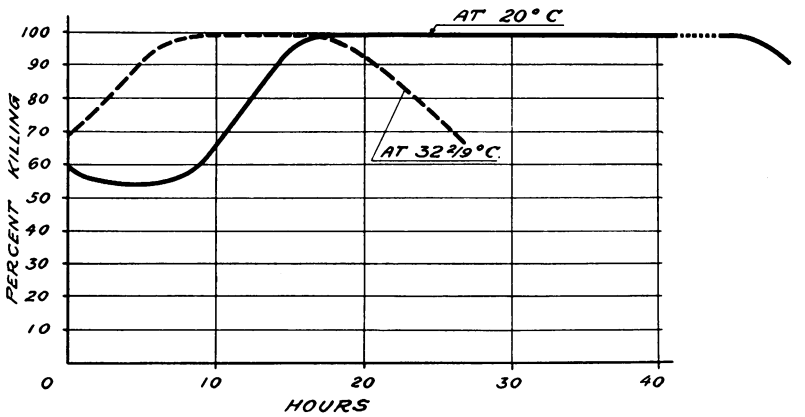


FIG. 7. THE RELATION BETWEEN THE PER CENT E. COLI KILLED AND TIME OF EXPOSURE FOR EQUAL AMOUNTS OF RADIATION APPLIED AT 20°C. AND 32⅔°C.

summarized in figure 8. It will be noticed that there are no improvements in the bactericidal action due to incubation for the short time exposures, i.e., for Petri plates nos. 1 to 6. On the contrary, incubation materially improved the lethal action for weak radiation for nos. 7 to 12. In considering plates 7 to 12 it must be remembered that these had progressively less exposure. Clearly, incubation shortened the lag period and hastened the growth or starting of the cells on their life cycle change and thus helped to bring the organisms to the sensitive state more quickly.

Taking advantage of the varying sensitivity of the organisms at different times of their life cycle it was shown that the same

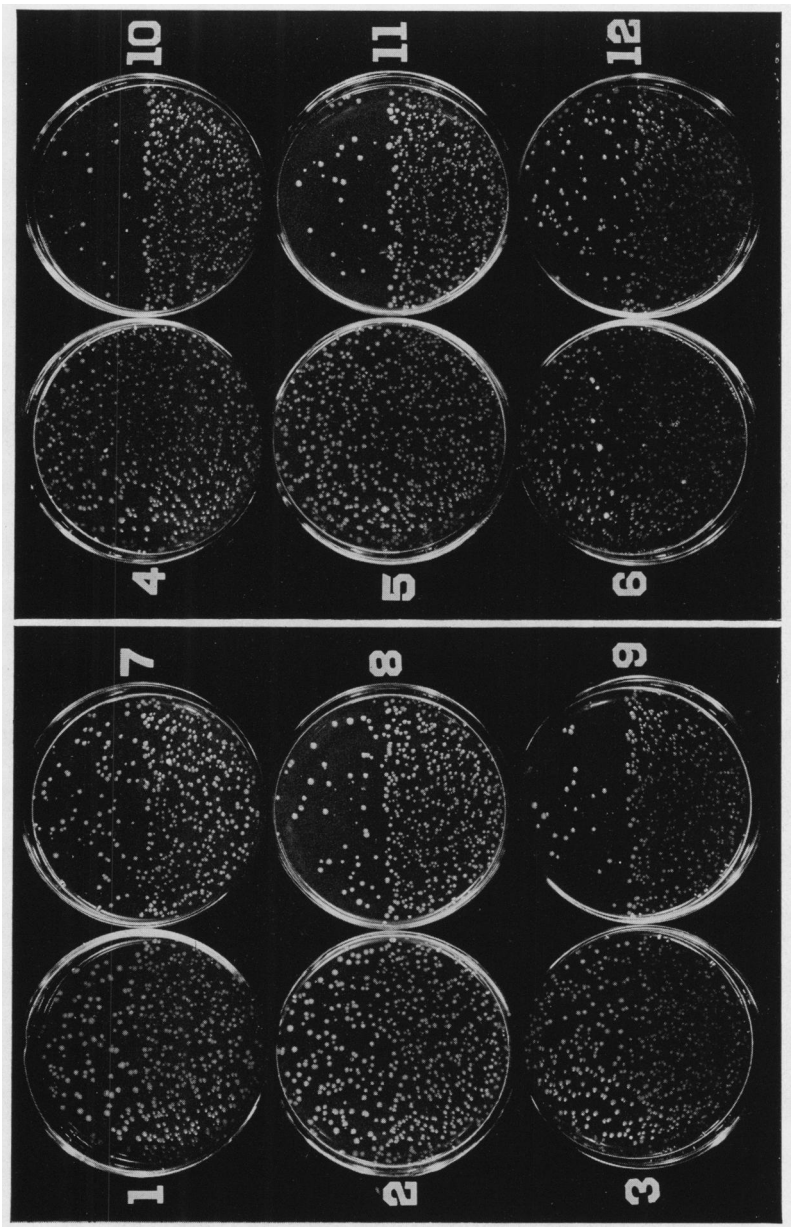


FIG. 8. EFFECT OF INCUBATION OF E. COLI BEFORE EXPOSURE TO ULTRAVIOLET RADIATION

#1 was exposed to a definite amount of radiation immediately after seeding. 7 was exposed to the same amount of radiation as #1 over a period of 11 hours. 2 incubated for 1 hour and exposed like #1. 8 incubated 1 hour and exposed like #7 for 10 hours. 3 incubated 2 hours and exposed like #1. 9 incubated 2 hours and exposed like #7 for 9 hours. 4 incubated 3 hours and exposed like #1. 10 incubated 4 hours and exposed like #7 for 8 hours. 5 incubated 5 hours and exposed like #1. 11 incubated 5 hours and exposed like #7 for 7 hours. 6 incubated 6 hours and exposed like #1. 12 incubated 6 hours and exposed like #7 for 6 hours.

results may be obtained from intermittent exposures as with continuous radiation extending over the same length of time (fig. 9).

Actual measurements show that it takes about five times as

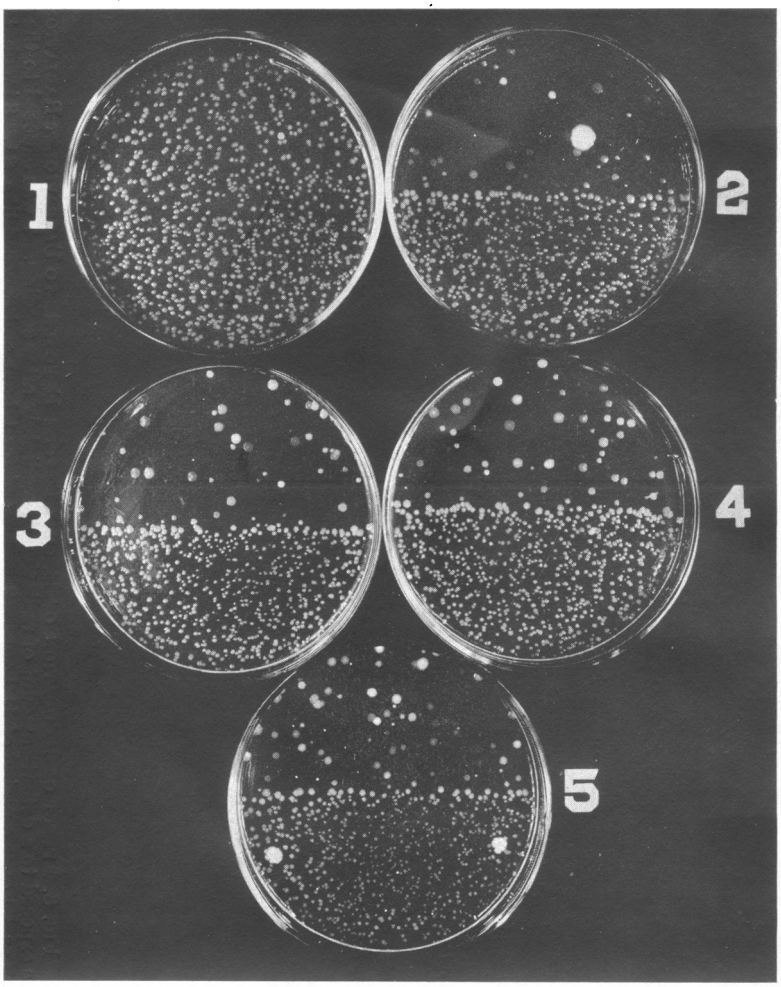


FIG. 9. BACTERICIDAL EFFECT OF INTERMITTENT VERSUS CONTINUOUS EXPOSURE ON E. COLI

*1, the total radiation was applied immediately after seeding; 2, the same total radiation was applied in 16 equal amounts at 1 hour intervals; 3, the same total radiation was applied in 8 equal amounts at 2 hour intervals; 4, the same total radiation was applied in 4 equal amounts at 4 hour intervals; 5, the same total radiation was applied by exposing to low intensity over a period of 16 hours.

much radiation as was applied to #1 in figure 6 to obtain the bactericidal action of #7, #8 or #9 in figure 6. This indicates that the variation in the sensitivity of the bacteria in a culture at the different stages of the life cycle is of the order of at least 5 to 1. Taking into consideration the lag period and other factors, leads to the conclusion that this ratio is definitely higher.

Further tests are necessary before one can determine the best relation between intensity, the time of exposure and the period of the life cycle of an organism to effect the destruction of all the individual cells with the least amount of radiation.

RESISTANCE TO ULTRAVIOLET RADIATION OF DIFFERENT INDIVIDUAL BACTERIA IN A CULTURE

Several Petri plates were seeded with *E. coli* in the usual manner but a dilute spray was used so that relatively few colonies appeared on incubation. This was done so that each colony would be most likely to originate from a single bacterium. A subculture was started by inoculating 25 ml. of sterile broth with a single colony from one of these plates. Five such subcultures, each from a different colony, were thus made. Each subculture was incubated for two hours at 37°C. and sprayed on plates in the usual manner. The spray box was thoroughly disinfected by ultraviolet radiation between the seedings of the different subcultures. The spray gun was sterilized before each spray. The different plates from each seeding were exposed to various measured amounts of radiation and incubated.

The bactericidal action for the different subcultures is given in table 8.

From the above table it appears that subculture "A" is relatively non-resistant while subculture "D" requires an abnormal amount of radiation for the same per cent of destruction of bacteria. Three subcultures, each from a single colony from one of the "A" plates were then incubated two hours and plates seeded in the usual manner. Three subcultures were similarly prepared from the "D" plates. These plates were exposed to different amounts of ultraviolet as shown in table 9.

All three subcultures from the "weak" culture, A, show a defi-

nitely higher percentage killed for exposures of 13 clicks or more than do the corresponding three subcultures from the resistant culture "D". From these results it appears that a resistant cul-

TABLE 8

Percentage bacteria killed in five subcultures each prepared from a different single colony of E. coli

ULTRAVIOLET EXPOSURE	A	B	C	D	E
clicks					
2	63	36	38.5	8.5	24.6
4	90.	43.	71.5	27.	20
13	99.4	86.	89.5	49.	81.6
20	99.6	93.4	97.2	74.	91
Number of colonies on controls.....	416	875	112	650	700

TABLE 9

Per cent colonies killed by varying amounts of ultraviolet in the three apparently low resistance subcultures from culture "A" of table 8 and in the similar three subcultures from the resistant culture "D"

U. V. EXPOSURE	PER CENT KILLED BY U. V. EXPOSURE					
	Three non-resistant subcultures from "A"			Three resistant subcultures from "D"		
clicks						
2	17	20	18	12	22	17
4	27	28	55	24	38	36
13	75	66	91	56	58	55
20	94	92	98.7	71	75	74
24	99.7	95	99.8	82	82	82
28	99.9	98	99.9	91	91	90
32	100.	99.7	100.	97	95	95
Colonies in controls..	1627	2640	1908	2610	2644	2670

ture can be grown from a resistant *E. coli* and similarly a less resistant culture from a weak one.

To determine the effect of a number of successive subcultures upon the resistivity to U.V. radiation of a given strain of *E. coli* the preceding experiment was repeated in a modified manner. A group of plates was seeded with *E. coli* and exposed to ultra-

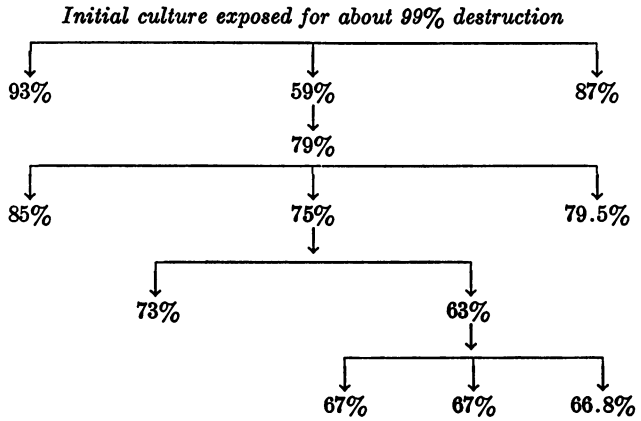
violet radiation so as to destroy about 99 per cent of the organisms. Three of the remaining colonies were incubated each in a separate broth for two hours at 37°C. and again seeded on Petri plates in the usual manner. These plates were exposed to 20 clicks of radiation and two-hour subcultures were made from some of the remaining colonies. The percentage destruction of a number of

TABLE 10

Per cent colonies killed in resistant and non-resistant strains of E. coli subcultured for 24 days

ULTRAVIOLET EXPOSURE	TWO NON-RESISTANT SUBCULTURES		TWO RESISTANT SUBCULTURES	
clicks				
2	49	47.7	11	31
4	71	80.8	47	37
7	94	97	60	52
10	99.5	99.5	66	64
13	99.5	99.9	76	79
17	99.9	100	85	74
20	100	100	90.5	87
24	100	100	95.5	95.5
28	100	100	98.3	97.8
32	100	100	99.2	99.2

such successive subcultures when exposed to 20 clicks of radiation are shown in the accompanying diagram.



A subculture was then made by inoculating sterile broth with a single colony from a non-resistant culture and similarly a second subculture from a resistant strain. Each was subcultured every third day for twenty-four days. Two sets of plates were then seeded from two-hour subcultures of the non-resistant strain and similarly two sets of plates from two-hour subcultures from the resistant strain. The percentage colonies killed by ultraviolet radiation from the two strains is shown in table 10.

From these tests it appears that in an average culture the individual bacteria are not only different in resistivity to ultraviolet radiation at different stages of their life cycle but that different individual bacteria are unlike in resistivity as well, and that they have the power to impart to an unmistakable extent this quality to a subculture.

RETARDATION OF BACTERIAL GROWTH BY ULTRAVIOLET RADIATION

The colonies on all the plates exposed to low intensity for a long period required a considerably longer time to develop upon incubation than was necessary for the control colonies on the same plates. This indicates a partial injury to the organisms that were exposed but not killed by the radiation. To obtain further information on the extent of the injury a number of Petri plates were seeded with *E. coli* and exposed to varying amounts of ultraviolet. The plates were all incubated at 25°C. instead of the usual 37°C. temperature and observed for the first appearance of colonies. (Judging from the appearance of the first visible colonies, the incubation of the exposed plate was retarded from two to six hours. Furthermore there is some indication that the life cycle of an organism exposed to radiation is appreciably lengthened.)

EFFECT OF HEAT UPON THE BACTERICIDAL ACTION OF ULTRAVIOLET RADIATION

The procedure was as follows:

(a) Of a resistant strain of *E. coli* 0.1 ml. of a 24-hour culture was mixed with 20 ml. of a sterile broth.

(b) Then 0.1 ml. of (a) was added to 200 ml. of sterile water.

1 ml. of (b) was plated in triplicate and incubated to determine the bacterial contamination of (b).

In a sterile test-tube 10 ml. of (b) were then heat treated by placing the test-tube in a water bath at 55°C. and constantly shaking it for a definite length of time. Simultaneously, a second tube with a similarly prepared non-resistant strain of *E. coli* was given this same heat-treatment, after which both tubes were rapidly cooled and 2 ml. of each plated in triplicate and incubated to determine the bacterial contamination surviving the heating.

A six minute heating to 55°C. reduced the bacterial count per ml. of the non-resistant strain by 98.8 per cent but only by 75 per cent for the resistant strain while a four minute heating reduced the count 67.8 and 57.7 per cent respectively. This shows that the strain of *E. coli* which is more resistant to ultraviolet radiation is also the more resistant to heat.

Of a 24-hour culture of a resistant strain of *E. coli* 0.5 ml. was then added to 10 ml. of sterile broth in a test-tube. The test-tube was placed in a water bath at 55°C. for 6½ minutes (this heating killed a large percentage of the *E. coli* as was shown above). To cool the culture 10 ml. of sterile broth at room temperature was then added to the test tube and Petri plates were at once seeded in the usual manner and the effect of ultraviolet radiation on the surviving organisms determined. The organisms surviving such a heat-treatment were materially less resistant to ultraviolet radiation for both the resistant and non-resistant strains. Upon subculturing however the normal resistivity of the strain was again recovered. This indicates a definite injury to the survivors of the heat treatment. Similar effects by Grenz rays and other agents are under investigation and the complete results will be reported in a later paper.

NATURE OF THE BACTERICIDAL ACTION OF ULTRAVIOLET RADIATION

The results reported in this paper have a definite bearing on the theories advanced for explaining the bactericidal action of ultraviolet radiation. The tests of the Reciprocity Law, for low

intensities show a far greater variation in the lethal radiation required to kill a bacterium at the different stages of its life cycle than was heretofore suspected. The increased lethal action for low intensities cannot be accounted for by the "single photon hit to kill" theory without making arbitrary assumptions as to the change in size of the vital spot during the life cycle. That different individual bacteria is an average culture such as *E. coli* may be materially different in resistivity to ultraviolet radiation and that this resistance characteristic is handed down to successive subcultures is important in analyzing test results on the bactericidal effects of ultraviolet radiation (the difference between individual cells naturally applies to cells in the same life cycle phase as, for example, when most resistant to radiation).

It is difficult to explain the apparent injury of those bacteria on a Petri plate not killed by the radiation (manifested by slower visible colony formation upon incubation) on the single photon hit theory.

The experimental data reported in this paper suggest that a bacterium is killed by ultraviolet radiation when it has absorbed the required amount of radiant energy in the lethal range (the amount depending upon the individual bacterium and the state of the organism at the time of irradiation), and that in any test there is a distribution of bacteria of different resistivities peculiar to that culture which distribution determines the relation between the bactericidal action and the amount of applied radiation. Clearly this does not explain how radiation changes the organism so that cell division becomes impossible. It, however, provides a guidance for the proper use of ultraviolet radiation as a bactericidal and fungicidal agent.

SUMMARY

1. A simple method is described for uniformly seeding the surfaces of Petri plates with bacteria or other microorganisms. This made possible a quantitative study of the bactericidal action of ultraviolet radiation under varied conditions with a reasonable number of tests.

2. An integrating ultraviolet meter which responds only to the

bactericidal wave band was devised. Errors in exposures due to fluctuations in light intensity and in character of the emitted radiation were thus eliminated.

3. The variation in the resistivity of the individual bacteria in a culture to ultraviolet radiation was shown to be considerably greater than was heretofore suspected. This explains many discrepancies generally attributed to other causes.

4. The Bunsen-Roscoe reciprocity law for the bactericidal action of ultraviolet radiation was proven by direct experiment for a range of intensities requiring only a few microseconds to several hours for producing the same amount of radiation. The law was found to break down when the time of exposure involves an appreciable part of the life cycle of the organism under condition of exposure.

5. The same individual organism was shown to have a widely different resistance to ultraviolet radiation at different stages of its life cycle.

6. In a normal culture of bacteria of a species such as *Escherichia coli* the resistance of different bacteria at the same stage of the life cycle to ultraviolet may be different.

7. The temperature of an organism at the time of irradiation has a negligible influence upon the lethal action of the ultraviolet radiation providing the temperature is such that there is no injury due to heat or cold.

8. A sublethal dose of ultraviolet radiation retards the rate at which colonies develop after irradiation.

9. From the data of tables 3 and 4 it appears that the average resistance to ultraviolet radiation of the individual cells of different types of bacteria is roughly of the same order of magnitude and that the apparent large variation is due to clumping and shielding.

10. The relation between the amount of ultraviolet radiation and the per cent of bacteria killed in a given culture is determined by the distribution of bacteria of different resistivity to the radiation and is not due to the probability of hitting a vital spot in a given organism by a photon.

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